# PATENT COOPERATION TREATY

# PCT

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference<br>0000053909 | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No.<br>PCT/EP 03/09369 | International filing date *(day/month/year)*<br>23.08.2003 | Priority date *(day/month/year)*<br>06.09.2002 |
| International Patent Classification (IPC) or both national classification and IPC<br>C12Q1/34 | | |
| Applicant<br>BASF AKTIENGESELLSCHAFT | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 04.03.2004 | 21.12.2004 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office - P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk - Pays Bas<br>Tel. +31 70 340 - 2040 Tx: 31 651 epo nl<br>Fax: +31 70 340 - 3016 | Authorized Officer<br>Tuynman, A<br>Telephone No. +31 70 340-3741 |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/09369

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

**Description, Pages**

1-31 as originally filed

**Sequence listings part of the description, Pages**

1-6 as originally filed

**Claims, Numbers**

1-19 received on 08.11.2004 with letter of 03.11.2004

**Drawings, Sheets**

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 19

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 19

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

☒ not complied with for the following reasons:

**see separate sheet**

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-17 |
| | No: | Claims | 18 |
| Inventive step (IS) | Yes: | Claims | 1-17 |
| | No: | Claims | 18 |
| Industrial applicability (IA) | Yes: | Claims | 1-18 |
| | No: | Claims | |

2. Citations and explanations

**see separate sheet**

**Re Item III**

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

Present claim 19 is and amended form of originally filed claim 20, for which no search report has been established for the following reasons: Originally filed claim 20 was part of a group of inventions for which no additional search fee has been paid (see item IV). Therefore no opinion shall be given on novelty, inventive step and industrial applicability of claim 19.

The reasoning for non-compliance with Rule 13.1 and 13.2 PCT as to unity of inventions is the following:

The present application (PA) relates to the provision of antifungal agents.

The special technical feature of method claim 1,7 and 9 (Rule 13.2 PCT) resides in an assay for determining GTP cyclohydrolase activity involving formate dehydrogenase. Neither the same nor a corresponding special technical feature (Rule 13.2 PCT) is present the method of culturing plants involving the use of inhibitors of GTP cyclohydrolase II (claim 19). No manufacturing relationship exists between the screening method of claim 1 and the inhibitor compounds used in the method of claim 19. Further the screening method is not a method of using the compounds used in claim 19. Therefore, there is no single general concept that links the screening method of claim 1 to the method of culturing plants of claim 19.
The provision of a further fungal GTP cyclohydrolase II according to claim 3 can be considered as a special technical feature (Rule 13.2 PCT) of independent claim 3. Therefore, independent claims 1,7,9 do not share a same or corresponding special technical feature (Rule 13.2 PCT) with independent claim 3 or 19. Independent claims 3 and 19 do not share a same or corresponding special technical feature either (Rule 13.2 PCT).
Therefore, unity of invention (Rule 13.1 PCT) is lacking a priori between the independent claims 3 and 19 and the group of independent claims 1,7,9.

Hence the examiner considers that the following separate inventions or groups of inventions are not so linked as to form a single general inventive concept:

1 Methods involving an assay for determining GTP cyclohydrolase activity involving formate dehydrogenase

2 A nucleic acid sequence of a further fungal GTP cyclohydrolase II (claim 3)
3 A method of culturing plants involving the use of inhibitors of GTP cyclohydrolase II (claim 19)

The groups of inventions 1 and 2 have been searched and shall form the object of an opinion as to novelty, inventive step and industrial applicability.

The applicant has not paid search fees for the subject 3 i.e. claim 19. In consequence the present examination is carried out on those claims for which a search has been carried out only, namely claims 1-18.

**Re Item IV**
**Lack of unity of invention**

See item III.

**Re Item V**
**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

Reference is made to the following documents:
D1: WO 00/40744 A (HERZ STEFAN ;BACHER ADELBERT (DE)) 13 July 2000 (2000-07-13)
D2: US-A-5 821 090 (BUITRAGO SERNA MARIA JOSE ET AL) 13 October 1998 (1998-10-13)
D3: BLAU N ET AL: BIOCHIMICA ET BIOPHYSICA ACTA, vol. 880, no. 1, 1986, pages 26-31.
D4: DATABASE WPI Section Ch, Week 198513 Derwent Publications Ltd., London, GB; Class B04, AN 1985-078062 & JP 60 030696 A (WAKO PURE CHEM IND LTD) 16 February 1985 (1985-02-16).

The document D1 is regarded as being the closest prior art to the subject-matter of independent claims 1,7 and 9 , and shows (the references in parentheses applying to this document):

1.1 D1, which is considered to be the closest state of the art with respect to independent claim 1, discloses that herbicides can be identified via a method comprising:

Incubating with a candidate compound a plant GTP cyclohydrolase II polypeptide and selecting a compound which reduces or blocks the activity of the GTP cyclohydrolase II (claim 1, claim 12; page 4, line 13-page 5, line 8; page 7, lines 19-21; page 14, lines 11-27). D1 suggests that antifungal agents can be identified via a similar method involving a fungal GTP cyclohydrolase II i.e. testing a candidate compound in a fungal GTP cyclohydrolase II inhibition assay. GTP is used as a substrate and the NADH content is monitored at 340 nm (page 14, lines 11-27). However, D1 does not provide a fungal GTP cyclohydrolase II.

Therefore the subject matter of independent claims 1 is novel.

1.2 With regard to independent claims 7 and 9, D1 is considered to represent the closest state of the art and discloses a method for the determination of GTP cyclohydrolase II activity and a method for the identification of inhibitors thereof (page 14, lines 11-22; page 4, line 21-page 5, line 8) from which the subject matter of claims 7 and 9 differs in that instead of the measurement of the 2,5-diamino-6-ribosylamino-4 (3H)-pyrimidine 5'-phosphate product of the reaction of GTP cyclohydrolase II, via a coupling reaction with 2,5-diamino-6-ribosylamino-4 (3H)-pyrimidine 5'-phosphate reductase and NADH, the formate product is measured using a coupling reaction of formate dehydrogenase and NAD. Note that in as far as in claims 7 and 9, cyclohydrolase I is involved D3 can be considered as the closest state of the art, which discloses yet a different assay for the measurement of GTP cyclohydrolase I.

Therefore the subject matter of independent claims 7 and 9 is novel.

1.3 D2 is considered to be the closest state of the art with respect to claim 3 and discloses a nucleic acid sequence encoding a fungal GTP cyclohydrolase II from Ashbya gossypii which differs from the nucleic acid sequence of claim 3 to such an extent ( i.e. a 69.4% identity is found in a 169 nucleotide overlap out of the 582 nucleotides of SEQ ID NO:4) that it does not fall under its scope.

Therefore the subject matter of independent claim 3 is novel.

1.4 With regard to independent claim 18, D1 is considered to be the closest state of the art, and suggests the use of a fungal cyclohydrolase as a target for the identification of antifungal agents (see item 1.1). However, D1 does not *de facto* disclose a fungal cyclohydrolase. Therefore, D1 is not considered as being

relevant with regard to novelty. The subject matter of claim 18 relates to the same subject matter and is supported by the description of the present application, which does *de facto* disclose a fungal cyclohydrolase.

2 The present application does not meet the requirements of Article 33(3) PCT, because the subject-matter of claim 18 does not involve an inventive step in the sense of Article 33(3) PCT.

2.1 From the analysis under 1.4 it can be concluded that the difference in subject matter between claim 18 and D1 can be considered to be the actual presence of a fungal cyclohydrolase for the intended use.

The problem to be solved may therefore be regarded as the provision of a fungal GTP cyclohydrolase. The proposed solution is a fungal GTP cyclohydrolase chosen from the group indicated on page 11, line 5-page 6, line 12.

The solution proposed in claims 1 of the present application cannot be considered as involving an inventive step (Articles 33(1) and (3) PCT) for the following reasons:

From D1 the person skilled in the art already knew that a fungal GTP hydrolase was a possible target for identifying antifungal targets. He will thus be prompted to see if a fungal GTP cyclohydrolase already has been disclosed. Without difficulty he will find D2 which indeed already had disclosed a fungal GTP cyclohydrolase II from Ashbya gossypii and expression systems therefore. The person skilled in the art certainly would include the enzyme of D2 in the assay of D1 thus arriving at a solution according to claim 18. It is noteworthy that the fungal GTP cyclohydrolase II from Ashbya gossypii of D2 fulfills the criteria of one of the preferred embodiments of the present description, page 5, lines 5-18.

3 The present claims 1-17 meet the requirements of inventive step, because their subject-matter involves an inventive step in the sense of Article 33(3) PCT.

3.1 With regard to independent claims 1,7 and 9, D1 is considered to represent the closest state of the art and discloses a method (see item 1.2) from which the subject matter of claims 1,7 and 9 differs most importantly in that instead of the measurement of the 2,5-diamino-6-ribosylamino-4 (3H)-pyrimidine 5'-phosphate product of the reaction of GTP cyclohydrolase II, via a coupling reaction with

2,5-diamino-6-ribosylamino-4 (3H)-pyrimidine 5'-phosphate reductase and NADH, the formate product is measured using a coupling reaction of formate dehydrogenase and NAD. The technical effect thereof is that instead of spectroscopically measuring NADH consumption at 340 nm, NADH production is measured.
Note that in as far as in claims 7 and 9, cyclohydrolase I is involved D3 can be considered as the closest state of the art, which discloses an assay for the measurement of GTP cyclohydrolase I.

The problem to be solved by the present invention may therefore be regarded as an alternative method for the determination of GTP cyclohydrolase (I or II) activity and a method for the identification of inhibitors thereof. The proposed solution is a coupling reaction of the formate product using formate dehydrogenase and NAD.

The solution proposed in claims 1,7 and 9 of the present application can be considered as involving an inventive step (Articles 33(1) and (3) PCT) for the following reasons:

The indirect bioassay through the 2,5-diamino-6-ribosylamino-4 (3H)-pyrimidine 5'-phosphate reductase mediated formation of NAD(P) is strongly dependent on the binding specificity between the indicator enzyme and the GTP cyclohydrolase. There is no clue in literature from adjacent technical fields (such as D4) that the formate dehydrogenase/NADH involving reaction can be used for the same purpose or has a suitable synergy with the GTP cyclohydrolase. Therefore to include this coupling reaction in the methods of claims 1,7 and 9 is not obvious to a person skilled in the art.

3.2 D2 is considered to be the closest state of the art with respect to claim 3 and discloses a nucleic acid sequence encoding a fungal GTP cyclohydrolase II from Ashbya gossypii which differs from the nucleic acid sequence of claim 3 to such an extent that it does not fall under its scope.

The problem to be solved may therefore be regarded as the provision of a further fungal GTP cyclohydrolase II. The proposed solution is a fungal GTP cyclohydrolase II (from Fusarium graminearum) according to the group indicated on page 11, line 35-page 6, line 12.

The solution proposed in claim 3 of the present application can be considered as

involving an inventive step (Articles 33(1) and (3) PCT) for the following reasons:

The sequences of prior art fungal GTP cyclohydrolases II differ to such an extent ( i.e. a 69.4% identity is found in a 169 nucleotide overlap out of the 582 nucleotides of SEQ ID NO:4) from the sequences falling under the scope of claim 3 that the person skilled in the art would have no staring point (i.e. primers to pick up a gene) to arrive at the solution of claim 3.

3.3 Claims 2,4-6,8,10-15 are dependent on claims 1,3,7 and 9, respectively and as such meet the requirements of the PCT as to novelty and inventive step.

3.4 Independent claims 16 and 17 involve a method according to claim 1,2,4-6,8 or 10-15 and as such meet the requirements of the PCT as to novelty and inventive step.

4 Present claims 1-18 fulfill the requirements of Article 33(4) PCT as to industrial applicability.

5 Further remarks:

Claim 7 mentions the determination of the "NAPH content" and claim 11 mentions the measuring of "NADPH" at 340 nm, whereas the description mentions the measuring of "NADH". In the present examination for these claims only "NADH" has been taken into account which is the normal product of formate dehydrogenase (D4, abstract). The claims should be corrected accordingly.

Amended Claims:

1. A method for identifying antifungal agents comprising
   i. incubating, with at least-one candidate compound, a fungal GTP cyclohydrolase II polypeptide under conditions allowing the binding of the candidate compound to the fungal GTP cyclohydrolase II; and
   ii. selecting, by step ii), at least a candidate compound which binds to the fungal GTP cyclohydrolase II of step i) ; or
   iii. selecting, by step iii) , at least one candidate compound which reduces or blocks the activity of the fungal GTP cyclohydrolase II of step i); or
   iv. selecting, by step iv), at least one candidate compound which inhibits or decreases transcription, translation or expression of the fungal GTP cyclohydrolase II of step i),

   whereby the GTP cyclohydrolase II activity in steps ii to iv is determined by

   a) adding GTP or GTP analog, $NAD^+$ and formate dehydrogenase to a sample comprising GTP cyclohydrolase II or I; and
   b) determination of the NADH content.

2. A method as claimed in claim 1, wherein the fungal GTP cyclohydrolase II is encoded by a nucleic acid sequence comprising
   a) a nucleic acid sequence shown in SEQ ID No: 1; or
   b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID No: 2 by back translation; or
   c) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from a functional equivalent of the amino acid sequence shown in SEQ ID No: 2, which has an identity with SEQ ID No: 2 of at least 49%, by back translation.

3. A nucleic acid sequence comprising
   a) a nucleic acid sequence shown in SEQ ID No: 4; or
   b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID No: 5 by back translation; or

c) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from a functional equivalent of the amino acid sequence shown in SEQ ID No: 5, which has an identity with SEQ ID No: 5 of at least 66%, by back translation.

4. A method as claimed in claim 1 or 2 which comprises testing a candidate compound in a fungal GTP cyclohydrolase II inhibition assay.

5. A method as claimed in claim 4 which comprises

a) incubating, with a candidate compound, a fungal GTP cy-clohydrolase II in a cell free system;

b) selecting, by step b), a candidate compound which decreases the activity of the fungal GTP cyclohydrolase II.

6. A method as claimed in claim 5, wherein the enzymatic activity of the fungal GTP cyclohydrolase II is determined in comparison to the activity of a fungal GTP cyclohydrolase II not incubated with the candidate compound.

7. A method for determination of GTP cyclohydrolase I or II activity comprising the steps of

a) adding GTP or GTP analog, NAD+ and formate dehydrogenase to a sample comprising GTP cyclohydrolase II or I; and

b) determination of the NAPH content.

8. A method as claimed in claim 5 or 6/ wherein the enzymatic activity of GTP cyclohydrolase II is determined according to claim 7.

9. A method for identification of inhibitors of GTP cyclohydrolase I or II comprising the following steps:

a) adding GTP or GTP analog, NAD+ and formate dehydrogenase to a sample comprising GTP cyclohydrolase I or II;

b) adding formate, NAD+ and formate dehydrogenase to a second sample comprising GTP cyclohydrolase I or II;

c) adding to the sample of step a) and step b) a candidate compound;

d) determining the [redacted]vity of both samples;

e) selecting candidate compounds that show inhibition in the presence of GTP and no inhibition in the presence of formic acid.

10. A method as claimed in claim 5 or 6, wherein inhibitors of fungal GTP cyclohydrolase II are identified in an inhibition assay according to claim 9.

11. A method as claimed in any of claims 5, 6, 8 and 10, wherein GTP is used as substrate and the NADPH content is determined by monitoring the increase in the absorption at 340nm.

12. A method as claimed in claim 1, 2 or 4 comprising the following steps:

a) the generation of organisms which, following transformation with a nucleic acid sequence encoding GTP cyclohydrolase II are capable of overexpressing polypeptide with GTP cyclohydrolase II activity;
b) the application/ to the organism of step a) and to an analogous, untransformed organism, of a candidate compound;
c) the determination of the growth, the viability or infectivity of the transgenic and the untransformed organism following application of the substance of step b) ;
d) the selection of candidate compounds, which reduces growth, viability or infectivity of the transgenic and the untransformed fungi following application of the substance of step b).

13. A method as claimed in claim 12, wherein the organism is a fungus.

14. A method as claimed in any of claims 1, 2, 4 to 6, 8 and 10 to 13, wherein the substances are identified in a high-throughput screening.

15. A method as claimed in any of claims 1, 2, 4 to 6, 8, 10 to 14, wherein the antifungal agent identified via the method is applied to a phytopathogenic fungus in order to verify the fungicidal activity.

16. A process for the preparation of a fungicidal composition, which comprises

a) identifying a antifungal agent via one of the methods as claimed in any of claims 1, 2, 4 to 6, 8 and 10 to 15, and
b) formulating the antifungal agent identified via (a), or an agriculturally useful salt of the active ingredient identified via (a), with suitable adjuvants.

17. A process for the preparation of a pharmaceutical fungicidal composition, which comprises

a) identifying an antifungal agent via one of the methods as claimed in any of claims 1, 2, 4 to 6, 8 and 10 to 15, and
b) formulating the antifungal agent identified via (a), or a pharmaceutically useful salt of the active ingredient identified via (a), with suitable excipients.

18. The use of a fungal GTP cyclohydrolase as target for the identification of antifungal agents.

19. A method for culturing plants or plant cells or plant tissues thereby controlling fungal growth comprising treating said culture with a fungicide, wherein said fungicide is a compound which is an inhibitor of fungal GTP cyclohydrolase II.